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TWO NEW METASTRONGYLE LUNG-WORMS FROM AUSTRALIAN MARSUPIALS.

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(With 6 Text-figures and Plates IV-VI.)

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During the last two years forty-five specimens of the common, shortnosed bandicoot, *Isoodon obesulus* Shaw and Nodder, have been examined for parasites. The bandicoots were collected in various Brisbane suburbs and at Mount Nebo, Mount Tamborine, Nambour, and Gympie. Lungworms were found in three specimens, all of which came from Indooroopilly, a suburb of Brisbane.

Two marsupial mice, *Antechinus flavipes* Waterhouse, were examined during the same period. One, which was collected at Mount Glorious, had a heavy infestation of lung-worms.

As far as can be determined, there are no previous records of lungworms in marsupials in Australia, although Travassos (1925) and (1946) described two lung-worms from South American opossums. The parasites recovered from the bandicoots and the marsupial mouse are sufficiently different from each other and from previously described species to warrant the erection of two new genera, for which the names *Marsupostrongylus* and *Plectostrongylus* are proposed.

The worms were fixed in hot 70% alcohol and preserved in 70% alcohol with 5% glycerine. Unless otherwise stated, examinations and measurements were made in lacto-phenol.

MARSUPOSTRONGYLUS n. gen.

GENERIC DIAGNOSIS: Metastrongylidae with delicate cuticle, which may be thrown into minute irregular ridges; no buccal cavity, oesophagus very short, clavate; intestine wide. Male with very small bursa, rays much reduced in size, anterior ray bilobed, lateral trilobed, externo-dorsal single, dorso-dorsal ray absent or represented by papillae. Spicules equal and similar, ending distally in membranous expansions; gubernaculum absent. Female with vulva immediately in front of anus, vagina short, with moderately developed muscular wall. Ovoviviparous. Parasites of the lungs of marsupials.

TYPE SPECIES : Marsupostrongylus bronchialus n. sp.

Marsupostrongylus is perhaps most nearly related to *Heterostrongylus* Travassos, from the lungs of South American opossums. It differs from it in having the spicules equal, in lacking a gubernaculum and in the absence of a dorso-dorsal ray. The bursa of *Heterostrongylus* is quite large, with well-developed dorsal rays, whereas in *Marsupostrongylus* it is small, with externo-dorsal rays short and the dorso-dorsal ray rudimentary.

It differs from *Plectostrongylus* n. gen. in general body form, in the strongly ornamented cuticle, in the blunt posterior end of the female, in the short vagina without a strongly developed ovijector, and in the absence of a gubernaculum in the male.

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MARSUPOSTRONGYLUS BRONCHIALUS n. sp.

HOST: Isoodon obesulus Shaw and Nodder, from Indooroopilly near Brisbane, South Queensland.

Holotype male and allotype female in the collections of the Queensland Museum.

HABITAT: The worms were orientated in the same way in all three infected bandicoots. They lay parallel to each other in the primary bronchus of each lung, with their posterior ends free in the lumen and their anterior ends penetrating the lung. As the primary bronchus was opened up, it was seen that some worms had entered each secondary bronchus, and as these in turn were opened up it was found that finally each female had penetrated singly into a bronchiole. The sharply tapered anterior end of each worm was pushed into a very fine bronchiole so that its head came to lie near the pleural surface of the lung.

The intestine of each worm was filled with brownish-black altered blood. Seen through the transparent cuticle, these dark tubes contrasted strongly with the milky-white uterine tubes (Plate IV). The males were usually lying free in the bronchi alongside the females, and their intestines were also filled with altered blood.

MALE: Length, 9 to 12 mm.; maximum width, 0.32 mm. at about 2 or 3 mm, from the anterior end. The body tapers towards each end, the width at the mouth is 0.036 mm., at the oesophageal-intestinal junction, 0.1 mm. and at the cloaca, 0.06 mm. The posterior end is slightly curved The cortical layer of the cuticle is extremely delicate and ventrally. voluminous. It has a tessellated appearance due to a mosaic of minute, irregular ridges. Some of these ridges have a definite transverse trend, giving the appearance of cross-striations in optical section. This layer is very loosely attached and is readily distorted during fixation and clearing. Labial and cephalic structures are inconspicuous; there appear to be 3 minute lips and 6 small papillae. The mouth leads into the oesophagus, which is slightly club-shaped and measures 0.23 mm. in length by 0.05 mm. in maximum breadth (cf. 2 Plate V, figs. 1 and 2). The testis is coiled near the anterior end, and the male duct passes back parallel to the intestine.

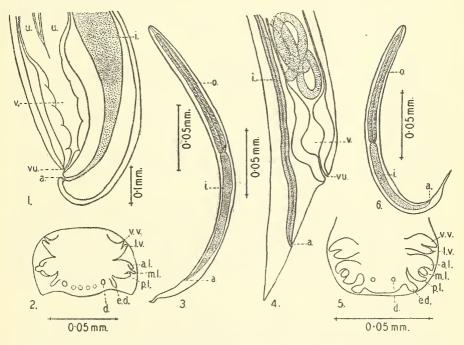
The bursa is small and delicate, with very short, stumpy rays (Plate V, fig. 5); the ventro-ventral and latero-ventral arise from a common trunk; the base of the lateral lobe is broad and relatively thick; the antero-lateral and postero-lateral rays appear as papilliform projections on either side of the medio-lateral ray; the externo-dorsal is single and rather slender; the dorso-dorsal ray is apparently represented by two papillae, one on either side near the base of the externo-dorsal ray; between these papillae there appears to be a row of four ill-defined papillae (Text-fig. 2).

The spicules are similar in size and shape measuring about 0.11 mm. by 0.01 mm. in maximum breadth. They are brown, narrow and curved. Proximally each ends in an irregular knob, while distally it expands into a membranous tip supported by three very delicate struts (Plate V, figs. 3 and 6). Neither gubernaculum nor telamon was detected.

FEMALE: Length, 20 to 35 mm.; maximum width, 0.8 to 1.0 mm. The body is fusiform, tapering very markedly anteriorly so that the anterior end somewhat resembles a well-sharpened lead pencil. The diameter at the mouth is 0.05 mm., at the oesophageal-intestinal junction, 0.19 mm. The width then increases very rapidly to 0.8 or 1.0 mm. at the region of the first uterine coils, which lie about 4 mm. from the anterior end. There is a distinct bulge at this point. The body then gradually narrows posteriorly, being about 0.5 mm. wide for the greater part of its length. It decreases to 0.27 mm. at the level of the junction of the uteri, and to 0.11 mm. at the vulva. The vulva is situated immediately in front of the anus which is 0.06 mm. from the rounded posterior end (Plate V, fig. 4; text-fig. 1).

The cuticle, mouth and oesophagus are similar to those of the male; the oesophagus measures 0.23 to 0.27 mm. by 0.05 to 0.06 mm. in maximum section. The nerve ring appears to be a little anterior to the mid-point of the oesophagus. The ovaries begin anteriorly and are thrown into several coils. The uteri, which are packed with developing ova, pass posteriorly parallel to the intestine. The uteri unite at about 0.3 mm. from the vulva. The vagina is relatively short, with moderately muscular walls. Ovoviviparous.

FIRST STAGE LARVA in uterus of female: Length, 0.23 mm. by 0.015 mm. in maximum diameter. There is a slender buccal cavity which leads into the oesophagus. This is 0.105 to 0.115 mm. long; it widens slightly posteriorly where it is about 0.006 mm. in diameter. The intestine and anus are well differentiated. The nerve ring lies 0.06 mm. from the anterior end, the tail ends in a sharply pointed, spur-like knob (Text-fig. 3).



Text-figs. 1-3. Marsupostrongylus bronchialus n. sp. 1. Posterior end of female 2. Male bursa; 3. First-stage larva.

Text-figs. 4-6. *Plectostrongylus fragilis* n. sp. 4. Posterior end of female; 5. Male burst, 6. First-stage larva. a. anus; a.l. antero-lateral ray; d. dorsal papilla; e.d. externo-dorsal ray; i. intestine; l.v. latero-ventral ray; m.l. medio-lateral ray; o. oesophagus; p.l. postero-lateral ray; u. uterus; v. vagina; vu. vulva; v.v. ventro-ventral ray.

PLECTOSTRONGYLUS n. gen.

GENERIC DIAGNOSIS: Metastrongylidae with smooth cuticle; body filiform; no buccal cavity; oesophagus short, simple, and slightly wider posteriorly. Male with very small bursa, rays much reduced in size, ventral ray bilobed, lateral trilobed, externo-dorsal single, dorso-dorsal ray absent or represented by papillae. Spicules equal and similar, ending distally in membranous expansions. Gubernaculum present. Female with posterior extremity straight, tail pointed; ovijector well developed; vulva anterior to anus and close to it. Ovoviviparous. Parasites of the lungs of marsupials.

TYPE SPECIES : Plectostrongylus fragilis n. sp.

Plectostrongylus resembles *Marsupostrongylus* in the bursal formula and having the spicules equal. It differs from it in the possession of a gubernaculum in the male; in the female the tail is pointed, and there is a long vagina ending in a muscular ovijector, whereas in *Marsupostrongylus* the tail is blunt and the vagina is relatively very short, with moderately muscular walls.

It differs from *Heterostrongylus* in having a greatly reduced bursa, in the absence of the dorso-dorsal ray, and in having the spicules equal and similar.

In the extreme reduction of the dorsal ray it seems to resemble *Pneumostrongylus* Mönnig from the impala, but differs from it in the reduced bursa, the bursal formula, the presence of a gubernaculum, the absence of a telamon. The eggs of *Pneumostrongylus* are segmenting when laid, whereas *Plectostrongylus* is ovoviviparous.

PLECTOSTRONGYLUS FRAGILIS n. sp.

HOST: Antechinus flavipes Waterhouse, from Mount Glorious, South Queensland.

Holotype male and fragments of females from the same host in the collection of the Queensland Museum.

HABITAT: The worms lay in the bronchioles, including the finer ones (Plate VI, fig. 7), and some even invaded the alveoli (Plate VI, fig. 6). The generic name indicates the intimate manner in which the worm is woven into the tissue of the lung.

MALE. One intact specimen, one complete but broken specimen and several fragments were obtained. The body is filiform, length 10 to 15 mm. by 0.088 mm. to 0.114 mm. in maximum diameter. The width at the oesophageal-intestinal junction is about 0.042 mm. Labia and cephalic papillae are inconspicuous. The cuticle is smooth. The mouth leads into the oesophagus, which is short, simple and slightly club-shaped, measuring 0.244 mm. in length by 0.02 mm. in maximum width. The intestine is a narrow tube.

The bursa is very small, and the rays are short and stumpy with a tendency to appear pedunculated (Plate VI, fig. 4). The ventro-ventral and latero-ventral arise from a common trunk, the ventro-ventral being the larger; these rays are incurved in all our specimens, but are shown diagrammatically as pointing outward in text-fig. 5. The antero-lateral and postero-lateral are broad and slightly larger than the medio-lateral; all the laterals arise from a common trunk; the externo-dorsal is short and blunt; the dorso-dorsal ray appears to be represented by two small papillae, one on each side near the base of the externo-dorsal ray.

The spicules are equal and similar. The proximal portion of each is broad, and the distal portion is drawn out into two fine rods, each surrounded by a membranous expansion. The spicules measure 0.09 to 0.1mm. in length by 0.01 mm. in maximum breadth; the membranous part is about 0.04 mm. long (Plate IV, figs. 2, 3 and 5). A V-shaped gubernaculum is present (Plate IV, fig. 2).

FEMALE. No intact specimens were recovered, so the length could not be determined. However, the fragments which were obtained suggested that the females are considerably longer than the males. The maximum width of some of the fragments is 0.1 mm. The width at the vulva is 0.04 mm. and the anus 0.026 mm. The posterior end is obliquely truncated, and the tail sharply pointed. The anus lies 0.036 to 0.05 mm., and the vulva 0.09 to 0.1 mm. from the tip of the tail (Text-fig. 4).

In one specimen, fixed in formol-acetic-alcohol and stained with haematoxylin, the vagina measures 0.83 mm. in length and terminates in a muscular ovijector. The uteri and vagina are packed with ova containing well-developed embryos. The ova measure 0.05 to 0.055 mm. by 0.026 to 0.03 mm.

FIRST-STAGE LARVA in uterus of female: Length 0.19 to 0.20 mm. by 0.008 to 0.01 mm. in width. There is a slender buccal cavity leading into the oesophagus, which is 0.09 to 0.095 mm. in length. The nerve ring is situated 0.05 mm. from the anterior end. The tail is sharply pointed (Text-fig. 6; Plate VI, fig. 1).

DISCUSSION.

The metastrongylid lung-worms have been studied recently by Dougherty, who has proposed a classification and discussed the evolution of the group (Dougherty, 1949, 1951). He has divided the family into six sub-families :—Metastrongylinae, Filaroidinae, Skrjabingylinae, Pseudaliinae, Protostrongylinae and Dictyocaulinae. The Filaroidinae (with six genera) have developed mainly in the Carnivora, the Pseudaliinae (four genera) in the Cetacea and the Protostrongylinae (ten genera) in the Artiodactyla. The Dictyocaulinae contains only one genus, *Dictyocaulus*, parasitic in the Ungulata, and bearing some striking resemblances to the Trichostrongylidae. The Skrjabingylinae (5 genera) occur in the Carnivora, Insectivora and one species, *Troglostrongylus delicatus* Travassos 1946, in the South American opossum. Dougherty places *Heterostrongylus*, the only other genus known from marsupials, in the sub-family METASTRONGYLINAE, together with *Metastrongylus* which is parasitic in pigs. *Heterostrongylus heterostrongylus* Travassos 1925 was described from the lungs of the South American opossum *Didelphis marsupialis aurita* Wied. It possesses a fairly large bursa with well-developed rays, although the arrangement of the dorsal system is unusual.

The two new genera described here cannot be definitely allotted to any of the above sub-families, although they appear to resemble some of the filaroidine genera in the reduction of the burss. We think that, until more material is studied from Australian marsupials and some of the lifehistories are elucidated, it is wisest to refrain from putting these genera into any of the known sub-families.

The hosts of our new species are polyprotodont marsupials belonging to different families. *Isoodon* belongs to the Peramelidae, which includes the bandicoots and rabbit-bandicoots, ground-dwelling, insectivorous or omnivorous creatures. *Antechinus* belongs to the Dasyuridae, which includes the carnivorous marsupials, the smaller members being mainly insectivorous. The South American opossums are also polyprotodonts, belonging to the family Didelphidae, arboreal creatures, mainly insectivorous or carnivorous.

It may be that the relationships of the parasites in the Metastrongylidae are a reflection of the *habits* of the hosts rather than of their phylogeny.

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SUMMARY.

Two new metastrongylid lung-worms are described, Marsupostrongylus bronchialus n. gen., n. sp., from the bandicoot, Isoodon obesulus, and Plectostrongylus fragilis n. gen., n. sp., from a marsupial mouse, Antechinus flavipes.

REFERENCES.

DOUGHERTY, E. C. (1949). The Phylogeny of the Nematode Family Metastrongylidae Leiper, [1909]: a correlation of host and symbiote evolution. Parasitology, 39, 222-234.

DOUGHERTY, E. C. (1951). A further revision in the classification of the Family Metastrongylidae Leiper [1909] (Phylum Nematoda). Parasitology, 41, 91-96.

TRAVASSOS, L. (1925). Un nouveau type de Metastrongylidae. Comp. rend. Soc. Biol., 93, 1259-1262.

TRAVASSOS, L. (1946). Rev. Brasil. Biol., 6, 499.

EXPLANATION OF PLATES.

Plate IV.

Marsupostrongylus bronchialus n. gen. and sp.

Bronchus of lung of Isoodon obesulus, opened to show the tails of lung-worms lying free in the lumen with their anterior ends entering the bronchioles. Note white uterine tubes and dark gut content of the worm.

Plate V.

Marsupostrongylus bronchialus n. gen. and sp.

Fig. 1. Anterior end of body of \mathcal{Q} . Fig 2. Cephalic region of same \mathcal{Q} showing oesophagus and tessellated cortical layer of the cuticle. Fig. 3. Side view of tail of \mathcal{J} . Fig. 4. Side view of tail of \mathcal{Q} . Note blood in the gut. Fig. 5. Side view of tail of \mathcal{J} showing the small bursa and rays. Fig. 6. Side view of tail of \mathcal{J} showing the tip of one spicule, which is extruded.

Scale. In fig. 1, one division of the scale is 0.05 mm. In figs. 2-6 inclusive each division of the scale is 0.01 mm. Figs. 2 and 4 are to the same scale.

Plate VI.

Plectostrongylus fragilis n. gen. and sp.

Fig. 1. First stage larva in smear made from fragments of an adult female. Fig. 2. Ventral view of tail of \mathcal{J} , showing the gubernaculum, bursa, extended membranous tips of spicules. Fig. 3. Lateral view of tail of \mathcal{J} , showing the spicules separated. Fig. 4. Lateral view of tail of \mathcal{J} , showing bursal rays. Fig. 5. Lateral view of tail of \mathcal{J} , showing spicules. Fig. 6. Section of lung of Antechinus flavipes showing cross sections of both \mathcal{J} and \mathcal{L} adult Plectostrongylus fragilis in the alveoli. Fig. 7. Section of lung of Antechinus flavipes showing cross sections of both \mathcal{J} and \mathcal{Q} adult Plectostrongylus fragilis in the alveoli. adult Plectostrongylus fragilis coiled up in a bronchiole.

The divisions of the scale represent 0.01 mm. in each figure. Figs. 2.5 are to the same scale; figs. 6-7 are to the same scale.



